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The Contribution of Transcriptomic and Proteomic Analysis in Elucidating Stress Adaptation Responses of *Listeria monocytogenes*

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Abstract

The foodborne transmission of *Listeria monocytogenes* requires physiological adaptation to various conditions, including the cold, osmotic, heat, acid, alkaline, and oxidative stresses, associated with food hygiene, processing, and preservation measures. We review the current knowledge on the molecular stress adaptation responses in *L. monocytogenes* cells as revealed through transcriptome, proteome, genetic, and physiological analysis. The adaptation of *L. monocytogenes* to stress exposure is achieved through global expression changes in a large number of cellular components. In addition, the cross-protection of *L. monocytogenes* exposed to different stress environments might be conferred through various cellular machineries that seem to be commonly activated by the different stresses. To assist in designing *L. monocytogenes* mitigation strategies for ready-to-eat food products, further experiments are warranted to specifically evaluate the effects of food composition, additives, preservatives, and processing technologies on the modulation of *L. monocytogenes* cellular components in response to specific stresses.

Introduction

Listeria monocytogenes is an important public health problem and food safety challenge. As a foodborne pathogen, this bacterium primarily targets immunosuppressed individuals, including the elderly, pregnant women, and newborns, leading to listeriosis, a disease that, although less frequent in occurrence (~2.4 cases/million), is associated with relatively high (averaging 20%–30%) mortality rates (Mead *et al.*, 1999). *L. monocytogenes* is associated with significant food safety control problems due to its wide distribution in nature and its capacity to survive and grow on the food products despite frequent exposure to harsh environmental conditions associated with food processing and preservation measures.

Upon encountering different environmental stress challenges along the food supply chain, it is presumed that *L. monocytogenes* cells depend on various stress-sensing mechanisms that detect stress-associated molecular hardships. On sensing such impending molecular stress problems, appropriate signal transduction processes are activated, subsequently leading to the mobilization of necessary stress protection measures through modifications in gene expression and protein function activities. Insights into gene expression changes mobilized during stress adaptation responses have been recently gained through transcriptome and proteome stress analysis in this bacterium. This article provides a review of cold, heat, osmotic, acid, alkaline, and oxidative stress responses in this bacterium.

Cold Stress Adaptation Responses of *L. monocytogenes*

Numerous molecular hardships confront *L. monocytogenes* cells exposed to cold stress such as increased membrane rigidity, reduced protein and enzyme activity, slow transport and nutrient uptake processes, stalled gene expression processes, and protein damage and alteration. Altered expression of numerous gene transcripts and proteins was observed in *L. monocytogenes* cells adapted to cold exposure (Chan *et al.*, 2007; Cacace *et al.*, 2010). Functional classification of genes and proteins identified in these two studies revealed that cold stress adaptation genetic responses of this bacterium promote (1) specific and general stress protection; (2) membrane fluidity and function; (3) resumption of gene expression events; (4) protein folding and degradation; (5) assimilation of carbon sources and cold protective nutrients; (6) oxidative stress protection; (7) energy production, and (8) specific amino acid and lipid biosynthesis pathways. A number of cold stress adaptation mechanisms that have been experimentally validated in this bacterium are listed in Table 1 and Figure 1A.

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Table 1. An Overview of the *Listeria monocytogenes* Gene and Protein Systems Associated with Cold, Osmotic, Acid, Heat, Oxidative, and Alkaline Stress Adaptation Responses

Stress adaptation gene/protein system	*Functional description*	*Phenotypically confirmed stress adaptation roles*						*References*
		Cold stress	*Osmotic stress*	*Acid stress*	*Heat stress*	*Oxidative stress*	*Alkaline stress*	
LisRK	Two component system	+	+	+	+			Cotter *et al.* (1999); Kallipolitis and Ingmer (2001); Sleator and Hill (2005)
KpdED	Two component system		+		+			Kallipolitis and Ingmer (2001)
Lmo 1172	Putative two component system protein	+						Chan *et al.* (2008)
Lmo 1060	Putative Two component system protein	+						Chan *et al.* (2008)
RsbV	Anti-antisigma factor protein.	+		+		+		Chaturongakul and Boor (2004)
RsbT	Serine kinase	+		+		+		Chaturongakul and Boor (2004)
SigB	Alternative sigma factor protein	+	+	+	+	+	+	Chan *et al.* (2007); Giotis *et al.* (2008a); Raengpradub *et al.* (2008)
SigC	Alternative sigma factor protein	+			+			Chan *et al.* (2008); Zhang *et al.* (2005)
SigH	Alternative sigma factor protein	+					+	Chan *et al.* (2008); Rea *et al.* (2004)
SigL/RpoN	Alternative sigma factor protein	+	+	+				Chan *et al.* (2008); Okada *et al.* (2006); Raimann *et al.* (2009)
MarR	MarR family transcription regulator						+	Rea *et al.* (2004)
Fur	Transcription regulator						+	Rea *et al.* (2004)
CtsR	Negative transcription regulator		+	+	+	+		Nair *et al.* (2000)
HrcA	Negative transcription regulator				+			Hu *et al.* (2007a)
MogR	Transcription repressor				+			van der Veen *et al.* (2009)
OppA	Oligopeptide permease	+						Borezee *et al.* (2000)
OpuC, Gbu	Osmolyte transporter	+	+					Angelidis and Smith (2003a, 2003b)
ProBA	Proline synthesis enzyme system		+					Sleator *et al.* (2001)
GAD	Glutamate decarboxylase system			+				Cotter *et al.* (2001)
Csps	Cold shock domain proteins	+	+			+		Loepfe *et al.* (2010); Schmid *et al.* (2009)
Hfq	RNA chaperone-	+	+					Christiansen *et al.* (2004)
Ctc	50S ribosomal protein L25		+					Gardan *et al.* (2003b)
HtrA	Serine protease		+	+	+	+		Wilson *et al.* (2006); Wonderling *et al.* (2004)
ClpB, ClpC, ClpE, ClpP	ATP dependent Clp chaperone and protease		+		+			Nair *et al.* (2000)
Fri	DNA binding protein of starved cells (Dps)	+			+	+		Dussurget *et al.* (2005)
Catalase	Catalase	+				+		Azizoglu and Kathariou (2010b)
SOD	Superoxide dismutase					+		Archambaud *et al.* (2006)
ADI	Arginine deiminase system			+				Ryan *et al.* (2009)
Lmo0038	Putative argmatine deiminase			+	+			Chen *et al.* (2009)
PgpH	Putative integral membrane and ppGpp hydrolase	+						Liu *et al.* (2006)
Lmo1078	Putative UDP glucose synthetase	+	+					Chassaing and Auvray (2007)
Bkd system	Branched chain a-keto acid dehydrogenase enzyme complex	+						Zhu *et al.* (2005)
F_0F_1-ATPase	Proton efflux membrane ATPase			+				Cotter *et al.* (2000)
LtrC	General stress response protein	+						Chan *et al.* (2007)
UvrR	DNA repair			+				Kim *et al.* (2006)

+ = Gene confirmed to have significant functional contributions under these stress conditions.

Readers interested in more detail on these cold stress adaptation systems are directed to recent review articles (Tasara and Stephan, 2006; Chan and Wiedmann, 2009). The subsequent discussion therefore only focuses on the cold stress response systems recently described in this bacterium and not covered by previous reviews.

The bacterial two-component systems (TCS) are part of signal transduction pathways with an important role in cold stress sensing. Deletions of *lisR* and two putative TCS (*lmo1172* and *lmo1060*) genes were recently shown to impair cold adaptation in this bacterium (Chan *et al.*, 2008). In addition, putative TCS genes *lhkA*, *yycJ*, and *yycF* have also been found to be transcriptionally activated in response to cold stress in *L. monocytogenes*, but their cold adaptation functional significance remains to be further investigated (Liu *et al.*, 2002; Chan *et al.*, 2007). The cold shock domain family proteins (Csps) and RNA helicases found in this bacterium are also important in cold growth functions. Csps are crucial in resolution of stabilized RNA secondary structures, which confront cold stress-exposed bacterial cells, leading to stalled transcription and translation events. Schmid *et al.* (2009) examined a series of *csp*-deleted *L. monocytogenes* mutants and found that mutants lacking *cspA* (*cspL*) and *cspD* genes were significantly impaired during cold growth. RNA helicases are also critical in RNA secondary structure resolution and thus vital to the resumption of transcription and translation events that get stalled under cold stress. The DEAD box RNA helicases are ATP-dependent RNA helicase proteins characterized by the presence of the D-E-A-D (Asp-Glu-Ala-Asp) amino acid sequence in the conserved helicase motif II. Three putative DEAD box RNA helicases (*lmo0866*, *lmo1722*, and *lmo1450*) were found to be part of the cold stress response transcriptome determined in *L. monocytogenes* (Chan *et al.*, 2007). Meanwhile, a transposon mutant disrupted in the *lmo0866* gene homolog of *L. monocytogenes* displaying significantly impaired cold growth was recently described further supporting the role of this putative RNA helicase protein in cold stress adaptation processes of this bacterium (Azizoglu and Kathariou, 2010a).

Heat Stress Adaptation Responses of *L. monocytogenes*

Proteins exposed to elevated heat stress are denatured, leading to aggregate formation and loss of function, including enzyme activity. Organisms adapting to heat stress must mobilize cellular mechanisms to restore membranes and nucleic acids functions, remove heat degraded proteins, and produce new proteins to restore metabolic functions lost through thermal stress destruction of proteins. Genome-wide transcriptome analysis has revealed that heat stress responses in *L. monocytogenes* involve the recruitment of various genes with roles in heat shock and SOS responses, as well as cell division and cell wall synthesis (Hu *et al.*, 2007a, 2007b; van der Veen *et al.*, 2007). This heat shock response is associated with increased production of a specific set of proteins, the heat shock proteins (Hsps), which includes highly conserved chaperones and ATP-dependent proteases, involved in damaged protein refolding and degradation. *L. monocytogenes* contains two heat shock stress response protein groups: the class I and III proteins that are negatively controlled through HrcA and CstR transcription repression mechanisms, and the σ^B controlled proteins, which include the class II stress response proteins, as well as class IV proteins with roles in general stress adaptation functions (Hu *et al.*, 2007a, 2007b). The HrcA repressor regulon includes chaperonin proteins GroES, GroEL, and DnaK. The activation of these proteins at elevated temperature provides cellular protection by assisting in protein folding and assembling (Hu *et al.*, 2007a). Further, the DnaK operon also includes the DnaJ and GrpE proteins, which are proposed to be important for stimulating the ATPase activity of DnaK (Liberek *et al.*, 1991). CstR repressor-controlled ATP-dependent proteases are ClpP, ClpE, and ClpC, which degrade damaged or misfolded proteins, and ClpB, which reactivates the protein aggregates (Hu *et al.*, 2007b). A *ctsR*-deleted mutant is more tolerant to heat stress consistent with CtsR in transcriptional repression of class III stress response gene expression (Hu *et al.*, 2007a). Table 1 and Figure 1B include various protein systems so far shown to contribute to heat stress adaptation responses of *L. monocytogenes*. These include genes of two component signal transduction systems (*lisR* and *kdpE*) as well as various negative (*hrcA*, *ctsR*, and *mogR)* and positive (σ^B and σ^C) transcriptional regulators (Kallipolitis and Ingmer, 2001; Zhang *et al.*, 2005; Hu *et al.*, 2007a, 2007b; van der Veen *et al.*, 2009).

Proteome analysis of heat stress responses in *L. monocytogenes* cells revealed increased production of GroEL and DnaK, which is consistent with the role of these highly conserved Hsps, in removal of altered proteins in *L. monocytogenes* cells exposed to heat stress (Sokolovic *et al.*, 1990; Morange *et al.*, 1993). Another heat stress response proteome study identified the general stress response protein Ferritin (also referred as ferritin like proteins, Flp) to be the predominant heat stress-induced protein in this bacterium (Hebraud and Guzzo, 2000). The *fri* gene transcripts were found to be heat stress inducible, whereas *fri*-gene-deleted *L. monocytogenes* cells had increased heat stress sensitivity (Hebraud and Guzzo, 2000; Dussurget *et al.*, 2005). Agoston *et al.* (2009) compared proteomes associated with mild and prolonged heat treatments on *L. monocytogenes* and discovered that a large number of metabolic proteins are suppressed by heat stress exposure. In addition, they also found that the Hsp DnaN, which is a beta subunit of DNA polymerase III, was highly induced in response to different heat shock treatments. Induction of DnaN as observed in this study thus suggests that *L. monocytogenes* also counteracts heat stress challenges by modulation of DNA replication processes apart from the elevated activity of protein folding and degradation mechanisms. Heat stress exposure also induces recruitment of SOS response and DNA repair genes, as well as PrfA-controlled virulence genes, while repressing the cell division and cell wall synthesis genes in line with suppressed cell division and cellular elongation observed under heat stress (van der Veen *et al.*, 2007).

Osmotic Stress Responses of *L. monocytogenes*

The high level of sugars and sodium salts typically used as food preservers threaten bacterial cells with dehydration, which leads to increased intracellular solute concentrations and disruption of various biological functions. Proteome analysis has been used to study osmotolerance responses in *L. monocytogenes*. Eleven osmotic stress acclimation proteins in this bacterium were identified, which include the GbuA, AppA, Ctc, DnaK, HtrA, and OpuC proteins (Duche *et al.*,

2002a, 2002b; Abram *et al.*, 2008). Specific osmotic stress adaptation protein systems currently known in this bacterium are listed in Table 1 and Figure 1C.

The accumulation of osmolytes constitutes one of the main osmotic stress adaptation strategies in *L. monocytogenes*. The BetL, Gbu, and OpuC transport systems, involved in glycine betaine and carnitine uptake, are important during growth of this bacterium under osmotic stress (Fraser *et al.*, 2003). The membrane-based and osmotically inducible K^+ uptake system, the Kdp-ATPase system, has been associated with osmotic stress protection roles in *L. monocytogenes* cells. The absence of the KdpE sensor and its regulator KdpD is asso-

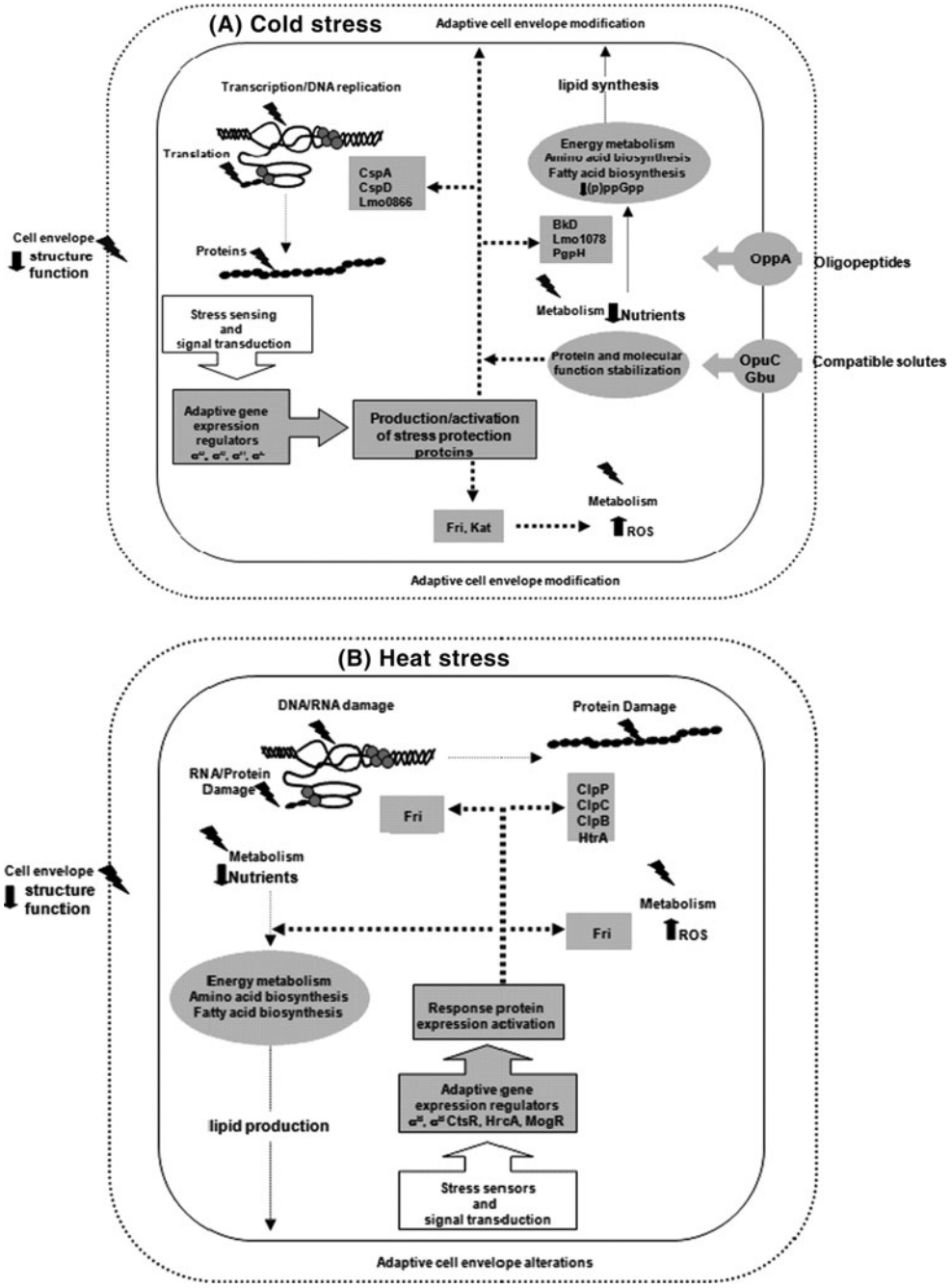

FIG. 1. Graphical presentations depicting stress-affected cellular components and a selection of molecular stress adaptation responses documented in *Listeria monocytogenes* cells exposed to **(A)** cold stress, **(B)** heat stress, **(C)** osmotic stress, and **(D)** acid stress. Alterations are caused by stress exposure on cell envelope, nucleic acids (RNA and DNA), proteins, and metabolism. Such molecular alterations are presumably detected by different stress-sensing molecular systems involving cell envelope components, nucleic acids, and proteins leading to signal transduction events and subsequent activation of adaptive gene expression response regulators. Gene expression modulation leads to activation stress protection functions through increased protein synthesis and/or activity modulation.

ciated with impaired *L. monocytogenes* growth under NaCl osmotic stress (Kallipolitis and Ingmer, 2001). Proline is also another osmolyte shown to be exploited for osmoprotection in *L. monocytogenes*. The disruption of the *proBA* locus encoding the ProA and ProB proteins was found to increase osmotic stress sensitivity of *L. monocytogenes* cells, indicating that proline biosynthesis genes may also be crucial in osmotic stress adaptation of this bacterium (Sleator *et al.*, 2001).

Single-gene deletions targeting *clpC*, *clpP,* and *htrA* genes are all also associated with defective cellular growth under NaCl osmotic stress tolerance in this bacterium (Wonderling *et al.*, 2004; van der Veen *et al.*, 2007). These observations thus suggest that these protein chaperones are also vital in resolution of protein damages and restoration of cellular functions that get impaired by osmotic stress. An *lmo1078*-gene-deleted *L. monocytogenes* EGD-e strain was described, which is also defective in growth under NaCl osmotic stress (Chassaing and Auvray, 2007). Lmo1078 is a putative UDP-glucose phosphorylase. This protein has been proposed to facilitate cell wall and membrane lipid composition maintenance, and its functions seem important during osmotic and cold stress adaptation of *L. monocytogenes* cells.

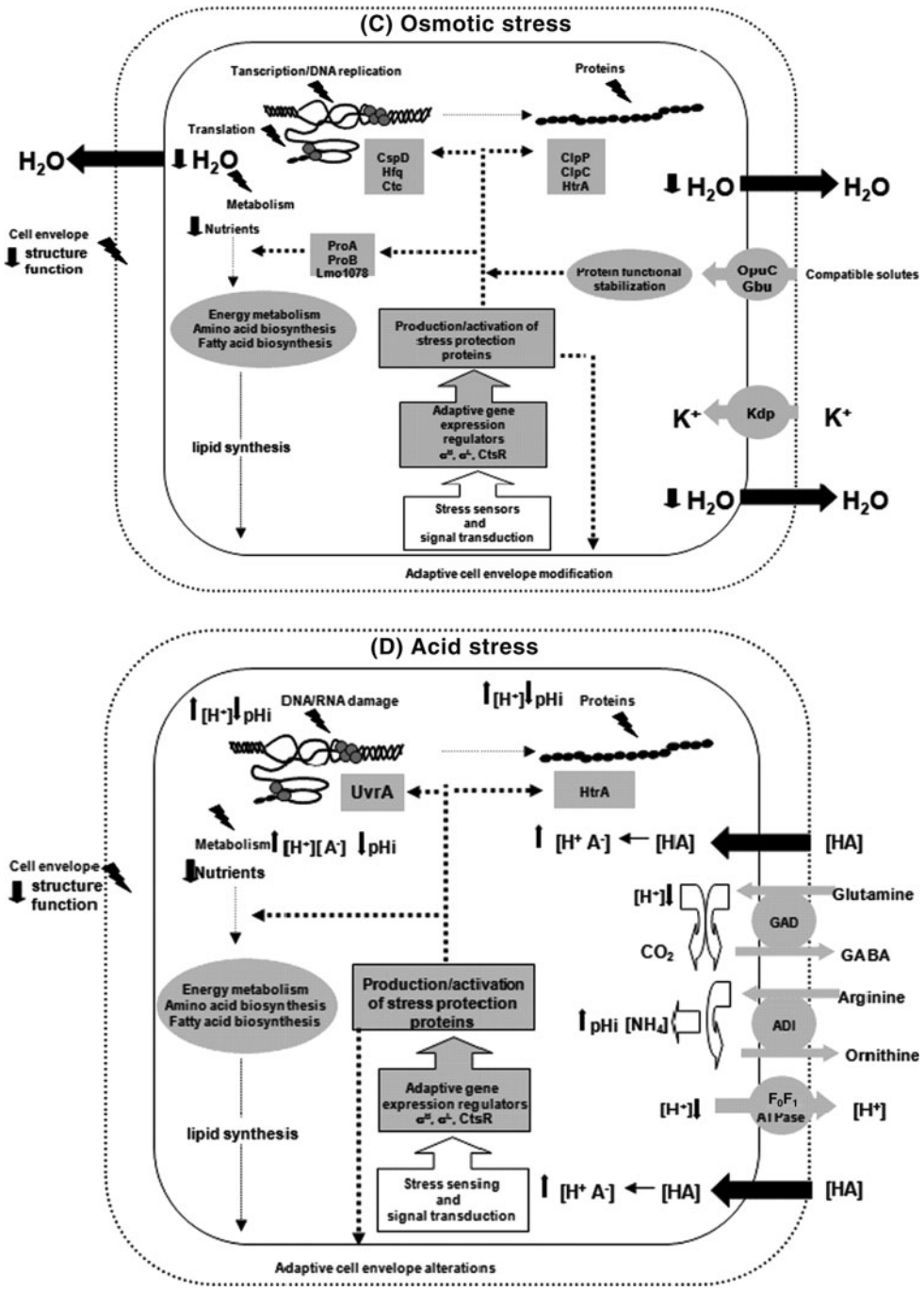

FIG. 1. *(Continued).*

Acid Stress Adaptation Responses of *L. monocytogenes*

The acid stress challenges faced by *L. monocytogenes* cells include organic acids used as food preservatives and decontaminants as well as inorganic acids encountered within the gastrointestinal tracts of human hosts. The antibacterial mechanisms against organic acid stress are generally presumed to depend on their dissociation in the bacterial cell cytoplasm, leading to acidification as well as proton and anion influx. Proton influx inhibits the cellular ATP synthesis and catabolite transport capacities, whereas the acidification and ionic influx into the cytoplasm disrupts metabolic functions as well as induce damage to proteins, nucleic acids, and cell membranes. Table 1 and Figure 1D list various acid stress response protein systems confirmed in this bacterium.

Bowman *et al.* (2010) recently examined transcriptome responses to organic acid stress in *L. monocytogenes*. They found that organic acid salt stress exposure (21 mM sodium diacetate at pH 5.0) was associated with a broad range of gene expression changes, including an increased activation of σ^B, PrfA, HrcA, and CtsR targeted regulons, as well as oxidative stress defenses, DNA repair, intermediary metabolism, cell-wall modification, and cofactor and fatty acid biosynthetic genes. A proteome analysis of *L. monocytogenes* ScottA cells exposed to food preservation organic acid salts also revealed an increased production of oxidoreductases and lipoproteins, and reduced expression of DNA-binding proteins, alpha amylase, and SecA (Mbandi *et al.*, 2007). The repression of DNA-binding proteins (involved in transcriptional regulation), alpha amylase (involved in metabolizing complex carbon sources in readily available energy sources), and SecA (involved in translocation of proteins) may therefore be interpreted as downregulation of cell metabolic activity caused by organic acid stress exposure of *L. monocytogenes* cells.

Other acid stress response proteomes induced mainly by exposure to hydrochloric acid have been determined in *L. monocytogenes* (Wemekamp-Kamphuis *et al.*, 2004; Phan-Thanh and Jansch, 2006). The identified acid stress proteins indicate that proteins involved in respiration (enzyme dehydrogenases and reductases), osmolyte transport, protein folding and repair, general stress resistance, flagella synthesis, and metabolism are all recruited as part of acid stress responses in *L. monocytogenes* cells. Cellular proton permeability involves altering the composition of the membrane lipid bilayer. Along these lines, increased straight chain fatty acid concentrations and decreased levels of branched chain fatty acids were observed in cell membranes of *L. monocytogenes* cells exposed to acid stress (Giotis *et al.*, 2007). Accelerated electron transfer through enhanced oxidation–reduction potential might be another mechanism employed by bacteria that are exposed to acid stress to dispel protons. The induction of dehydrogenases (GuaB, PduQ, and Lmo0560), reductases (YcgT), and respiratory enzymes associated with acid stress responses in *L. monocytogenes* cells may therefore also indicate acid stress adaptive mechanisms, which involve active cellular proton efflux (Phan-Thanh and Jansch, 2006). Along the same lines, the F_0F_1 ATPase also plays an important role during *L. monocytogenes* acid stress adaptation, where it is thought to contribute to proton efflux events (Cotter *et al.*, 2000).

The glutamate decarboxylase (GAD) acid resistance system serves important acid stress adaptation roles in this bacterium (Cotter *et al.*, 2001; Wemekamp-Kamphuis *et al.*, 2004). The *L. monocytogenes* arginine deiminase (ADI) system includes enzymes encoded by *arcA*, *arcB*, and *arcC*, which are involved conversion of arginine to ornithine, as well as the antiporter *arcD*, which transfers ornithine outside the cell in exchange for arginine (Ryan *et al.*, 2009). An *argR* and *arcA* deletion mutant showed impaired survival under mild (pH 3.5) and lethal (pH 4.8) acid stress conditions, respectively. The *lmo0038*, encoding a putative peptidylarginase deiminase family gene, has also been suggested to have acid stress adaptation functions in this bacterium (Chen *et al.*, 2009). The TCS LisRK has been linked to acid stress response modulation probably through its role in sensing of acid stress-related environmental stimuli (Cotter *et al.*, 1999; Sleator and Hill, 2005). In addition, *L. monocytogenes* cells lacking HtrA are acid stress sensitive consistent with the requirement of HtrA protease functions in removal of acid stress-damaged proteins (Wonderling *et al.*, 2004).

L. monocytogenes Response to Alkaline Stress

Environmental *L. monocytogenes* strains inhabiting food-processing environments may frequently encounter sublethal alkaline stress associated with detergents and sanitizing agents applied as food hygiene measures. The adaptive strategies deployed in microorganisms during alkaline stress adaptation include metabolic changes to increase acid generation, as well as the induction of transporters and enzymes involved in proton retention, and cell surface modifications that promote cytoplasmic proton retention. Transcriptome analysis has revealed that as much as 390 gene transcripts in *L. monocytogenes* cells are differentially expressed in response to alkaline stress exposure. Genes mobilized for alkaline stress adaptation participate in general stress responses, solute transport, and various metabolic pathways (Giotis *et al.*, 2010). In another study the proteome analysis of *L. monocytogenes* cells exposed to alkaline stress revealed the synthesis or over expression of a limited number of proteins as compared to the repressed expression of proportionally large number of proteins (Giotis *et al.*, 2008b). Meanwhile, the stress protective chaperones DnaK and GroEL were also induced by alkaline stress exposure. By screening of a library of Tn*917- lac* insertional mutants in *L. monocytogenes* LO28, Gardan *et al.* (2003a) identified 12 mutant strains that were sensitive to alkaline stress, and these mutants were disrupted in genes encoding putative transporter proteins. At present there is, however, still less known about specific alkaline stress adaptation protein systems in this bacterium.

Oxidative Stress Adaptation Responses

L. monocytogenes cells must combat oxidative stress encountered in external environments and during host infection. In food environments, oxidative stress might develop due to atmospheric modification as well as by chemical reagents applied as detergents and disinfectants. Reactive oxygen species (ROS) are also produced as byproducts of metabolism and can accumulate due to respiratory chain impairment or metabolic alterations encountered in other stress situations (Bowman *et al.*, 2010; Cacace *et al.*, 2010). Oxidative stress is deleterious to various molecular processes and cellular components such as membranes, proteins, nucleic acids, and enzymes. To cope against oxidative stress, molecular

detoxification of ROS, as well as protein, membrane, and nucleic acid damage repair mechanisms must be activated.

Gorski *et al.* (2008) found that best colonizing *L. monocytogenes* strains in oxidative food environments were those with an enhanced ability to tolerate oxidative stress exposure. Bacterial ROS detoxification systems include superoxide dismutase, catalase (Kat), and alkyl hydroperoxide (AhpCF) enzyme systems (Helmann *et al.*, 2003). The Kat and superoxide dismutase have been shown to be important in oxidative stress protection of *L. monocytogenes* cells. The *sod* and *kat* null *L. monocytogenes* strains display poor aerobic growth phenotypes as well as increased oxidative stress sensitivity, poor macrophage survival, and virulence (Archambaud *et al.*, 2006; Azizoglu and Kathariou, 2010b). The Dps ferritin has also been shown to be important in oxidative stress protection of *L. monocytogenes*. The cells of this bacterium that are deleted in the ferritin protein encoding gene *fri* exhibit increased sensitivity to oxidative stress and poor macrophage infectivity (Dussurget *et al.*, 2005). A ferric transcriptional regulator *per* deleted *L. monocytogenes* mutant was reported to exhibit increased sensitivity to hydrogen peroxide and poor aerobic growth (Rea *et al.*, 2005). In addition, Per regulon determination in this study unveiled Per-dependent repression of numerous ROS detoxification genes, including the *kat*, *trxB*, *fur*, *fri*, and *hemA* genes. The increased peroxide sensitivity in *per* mutant cells was suggested to be a potential consequence of increased expression of some ROS defense genes that are otherwise normally controlled through Per repression.

Role of σ Factors in *L. monocytogenes* Stress Adaptation

Proteins encoded and governed by global general stress response regulator σ factors play vital roles in protecting *L. monocytogenes* cells against various stress stimuli. σ^B is a positive regulator of several general and cold stress response genes, including *fri*, *oppA*, *opuCA*, and *ltrC* genes, with genetically confirmed cold stress adaptation roles in this bacterium (Chan *et al.*, 2007). While recently defective cold growth phenotypes were also documented in *L. monocytogenes* mutant strains lacking genes for the σ^C, σ^H, and σ^L (RpoN) alternative sigma factors, indicating that besides σ^B, these alternative sigma factors are also involved in regulation of cold adaptation processes in this bacterium (Chan *et al.*, 2008; Raimann *et al.*, 2009). The *L. monocytogenes* class II Hsps are positively controlled by σ^B, and increased heat stress sensitivity in a σ^B deletion mutant is consistent with σ^B-dependent transcriptional activation of the class II stress response genes in this bacterium (van der Veen *et al.*, 2007). Apart from σ^B, the loss of σ^c function also increases *L. monocytogenes* sensitivity to a thermal treatment (Zhang *et al.*, 2005).

Both σ^B and σ^L in *L. monocytogenes* are transcriptionally upregulated in response to elevated NaCl concentrations, whereas σ^B- and σ^L-deleted mutants of this bacterium are NaCl salt-stress sensitive (Okada *et al.*, 2008; Raimann *et al.*, 2009). Based on transcriptomic profiling, σ^B-regulated genes in osmotic stress adaptation are associated with general stress responses, transcriptional regulation, cell transport, envelope modification, metabolism, protein synthesis and modification, as well as virulence-associated functions (Raengpradub *et al.*, 2008). Genes positively controlled through σ^B also include osmolyte transporter genes (*opuCA* and *gbuA*) as well as *clpC* and *hfq* genes, which have all been genetically associated with osmotic stress adaptation (Christiansen *et al.*, 2004; Abram *et al.*, 2008; Raengpradub *et al.*, 2008). The general stress response protein Ctc is vital in NaCl salt stress tolerance and the *ctc* gene in *L. monocytogenes* is preceded by a putative σ^B-dependent promoter (Gardan *et al.*, 2003b).

Both σ^B and σ^L have also been functionally linked to regulation of the acid stress adaptation responses in this bacterium. *L. monocytogenes* cells deleted on σ^B and its regulators *rsBT* and *rsBV*, as well as σ^L, have been found to display acid stress sensitive phenotypes (Chaturongakul and Boor, 2004; Wemekamp-Kamphuis *et al.*, 2004; Raimann *et al.*, 2009). σ^B is also a positive regulator of genes of the GAD and ADI acid stress response systems and its deletion diminishes expression of GAD and ADI genes (Cotter *et al.*, 2001; Ryan *et al.*, 2009). σ^B regulons in this bacterium have also been shown to include several oxidative stress response genes and σ^B-deleted mutant strains are significantly impaired in oxidative stress tolerance (Ferreira *et al.*, 2001; Hain *et al.*, 2008; Oliver *et al.*, 2010). It has been recently described that the σ^B contribution to oxidative stress might also depend on strain genotype. Oliver *et al.* (2010) found that although σ^B loss induced oxidative stress sensitivity in strains of lineage I, II, and IIIB, this was not the case in a lineage IIIA strain. Such differences between the *L. monocytogenes* genomic lineages lead to a conclusion that such strain-specific differences might therefore also influence σ^B-dependent oxidative stress response gene regulation, in different genomic lineages of this bacterium.

Cross Protective Stress Responses

It has been shown through several studies that the exposure of *L. monocytogenes* to sublethal stress induces the development of stress-conditioned organisms, which are physiologically more tolerant to increased levels of the same or different stresses. For example, *L. monocytogenes* cells exposed to sublethal acid stress display increased resistance to higher acid stress levels as well as become more tolerant to heat and osmotic stress (Gahan *et al.*, 1996). Acid- and cold-adapted cells of this bacterium are more protected from the effects of high hydrostatic pressure stress (Wemekamp-Kamphuis *et al.*, 2002). *L. monocytogenes* cells preadapted by exposure to sublethal heat stress show enhanced osmotic and ethanol stress protection (Lou and Yousef, 1997). Taormina and Beuchat (2002) showed that alkaline stress-adapted *L. monocytogenes* cross-contaminating food products are more resistant to thermal food safety measures in comparison to those not previously exposed to sublethal alkaline stress conditions.

To date, investigators have identified various molecular mechanisms that appear to be crucial in mediation of cross protective stress responses in this bacterium. For instance, molecular adaptive challenge envisaged in cold environments, particularly freezing, include intracellular accumulation of ROS due to cold stress-induced metabolic alteration. A catalase-deficient *L. monocytogenes* F2365 mutant was recently described, which is impaired during cold growth (Azizoglu and Kathariou, 2010b). In addition, mutants lacking some *csp* genes are also characterized by increased peroxide stress sensitivity and reduced host cell infectivity (Loepfe *et al.*, 2010). Moreover, *L. monocytogenes* mechanisms to combat osmotic stress seem to also involve functions of the cold shock domain family proteins. A diminished osmotic stress

tolerance phenotype is induced by *cspD* deletion in this bacterium (Schmidt *et al.*, 2009). A *cstR* null mutant was described vital to osmotic stress adaptation responses of *L. monocytogenes* cells, indicating that some CtsR-repressed heat stress proteins also contribute toward osmotic stress (Nair *et al.*, 2000). Another heat stress repressor protein, HtrA, is also presumed to degrade misfolded or aggregated proteins that accumulate when *L. monocytogenes* cells are exposed to harsh environmental conditions such as high osmolarity and low pH in addition to high temperatures (Wilson *et al.*, 2006; van der Veen *et al.*, 2007). Giotis *et al.* (2008b) reported induced expression DnaK and GroEL chaperones by alkaline stress and as such these proteins have very well-defined role in heat stress adaptation. Thus, based on above examples it is evident that a large number of proteins and regulatory mechanisms are involved in *L. monocytogenes* cross protective responses.

Conclusion and Future Perspectives

The development of more effective food preservation methods depend on an improved understanding of fundamental changes that are instituted at the gene expression level in cells of this bacterium when challenged with adverse environmental stress conditions (Tasara and Stephan, 2006). Although considerable progress has been made so far in understating the *L. monocytogenes* stress response, one major draw back is that the majority of current information is based on broth models and not with actual food substrates. We therefore propose that more efforts should be invested in understanding the cellular stress response of *L. monocytogenes* at the molecular level in the presence of different food substrates and varying environmental conditions. It is hoped that with this further knowledge, the complexity and hierarchical nature of stress adaptation response mechanisms can be understood better, paving the way to the development of novel strategies that are more effective in combating the stress resistance properties of *L. monocytogenes*. Such future breakthroughs may include (1) novel ways to block the expression of transporter proteins that govern cold/osmotic stress adaptation to prevent the uptake of osmolyte molecules from food substrates to make *L. monocytogenes* cells susceptible to both cold and osmotic stress stimuli, and (2) identifying the antimicrobial compounds that suppress the induction of stress proteins of *L. monocytogenes* in food products.

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